# The Potential for Ethanol Production From Alfalfa Fiber Derived From Wet Fractionation

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# Introduction

Wet fractionation of forage crops allows biomass to be produced at very competitive prices due to the high values of the coproducts. The fractionation process consists of expressing juice from fresh herbage. The resulting fibrous fraction is high in cell wall constituents (cellulose, hemicellulose and lignin). It can be immediately field-dried and baled or pelleted if desired to minimize handling, transportation and storage costs. It is suitable for combustion, gasification, or enzymatic hydrolysis and fermentation to ethanol.

The juice fraction typically contains 25 to 30% of the dry matter in the original herbage depending on the severity of processing. It is high in protein and solubles and is almost fiber free. It can be used to produce both food-grade and feed-grade protein concentrates as well as other high-value products. The anticipated market value of the juice products, based on current prices of analogous products, greatly mitigates the production costs of the biomass fraction.

Wet fractionation of green herbage, the separation of fiber and juice, to produce protein concentrates has been researched and developed for more than a half century (Telek and Graham, 1983; Pirie, 1987). The technology and products are well known. For most of this period, however, emphasis was on making a crude, green protein concentrate either for livestock feeding or for supplementing the diets of humans suffering from protein deficiency. While the nutritional value of this product was undisputed, the economics of the process were, at best, marginal. Therefore, it was not, in general, commercialized.

Subsequent developments, however, appear to have greatly improved the potential for profitability:

- 1. Researchers have identified high-value juice products including soluble protein with good functional properties for human food (Knuckles and Kohler, 1982; Kohler et al., 1983), xanthophyll concentrates for use in the poultry industry (Crombie, 1995) and other products such as plant and animal growth stimulants, cosmetic substances, and pharmaceuticals (Koganov, 1992; Koganov et al., 1988).
- 2. Biotechnologists at the University of Wisconsin have demonstrated the possibility of adding genes to alfalfa which cause the transgenic alfalfa to produce industrially valuable substances, especially enzymes. Fields of alfalfa could thus become "bioreactors" or "enzyme factories" with the target enzymes recovered from the juice.

To date, transgenic alfalfa cultivars containing manganese dependent lignin peroxidase for biopulping, α-amylase for converting starch to sugar, cellulases for saccharification of lignocellulosics, and phytase to allow poultry and swine to utilize otherwise insoluble phosphorus in their grain-based rations, respectively, have been produced.

The use of forage crops, especially perennial legumes as a source of biomass, has a number of other advantages:

- 1. The need for nitrogen fertilizer, a high energy non-renewable input, is eliminated. Infrequent reestablishment of the crop minimizes the energy requirement for tillage and seed bed preparation.
- 2. Biomass can be field-dried and pelleted or cubed to minimize transportation, handling, and storage costs.
- 3. Forage varieties adapted to a wide range of environmental conditions exist, and production practices are established.
- 4. Machinery and methods for production and harvesting are available.

5. The excellent soil and water conservation characteristics of perennial forage crops are well recognized. This makes their production not only sustainable, but also desirable.

The economics of biomass production via wet fractionation is dependent on both unit prices and per hectare production of the various fractions.

#### Methods

In the spring of 1995 at Madison Wisconsin, four plots of approximately 42m<sup>2</sup> each were established in an alfalfa field which had been seeded the previous year. The first plot was mowed on May 22 and May 21 in 1995 and 1996, respectively, with successive plots mowed at 8-9 day intervals. This was done to "stage" the area as would be necessary in a large scale operation where it is required to keep harvesting and processing equipment working steadily at near capacity during the entire growing season. Each plot was then harvested at approximately 35-day intervals after its initial harvest for a total of four harvests per plot. The herbage from each plot was weighed and immediately macerated using a double rotor, rotary impact macerator (Koegel and Straub, 1994). Juice was then expressed by running the macerated herbage twice through a 15 cm diameter Rietz screw press. Herbage, juice, and fibrous fraction samples were oven-dried at 105 °C for 24 hours to determine dry matter content.

The following analyses were carried out on the fibrous fraction: (1) neutral detergent fiber (NDF), (2) acid detergent fiber (ADF), (3) acid detergent lignin (ADL), (4) nitrogen, and (5) ash.

The following definitions were used: (1) hemicellulose = NDF - ADF, (2) cellulose = ADF - ADL, (3) lignin = ADL, and (4) protein = 6.25 x nitrogen. Solubles were determined by difference.

### Results

Per hectare dry matter yields of herbage and juice for 1995 are 13.5 t/ha and 4.2 t/ha,

respectively, for an overall juice:herbage DM ratio of 0.31. In 1996, herbage dry matter yield was 12.9 t/ha and juice DM yield was not measured.

Table 1 gives the composition of the biomass or ligno-cellulosic fraction resulting from wet fractionation of the four harvests of each of the four plots in terms of cellulose, hemicellulose, lignin, protein, ash, and solubles (by difference). These are also converted to per hectare yields based on 9.45 t/ha of fibrous fraction.

Table 2 shows the <u>potential</u> per hectare production of ethanol based on yields of 85% of the stoichiometric. The soluble dry matter, which has not been characterized, was assumed to yield at the same rate as cellulose.

Wyman et al. (1993) give ethanol yields (l/t) of cellulosic biomass as 338 for "reference case" and 497 for "improved technology." Multiplying these yields times the 9.45 t/ha of alfalfa fibrous fraction gives 3194 l/ha and 4697 l/ha, respectively, which bracket the 4200 l/ha shown in Table 2.

Wyman et al. (1993) give approximate compositions of three classes of lignocellulosics: "agricultural residue," "hardwoods" and "herbaceous plants." The ratios of hemicellulose to cellulose for these three groups are approximately 1:1.2, 1:2.2, and 1:1.5, respectively, and the sums of hemicellulose plus cellulose are 70%, 73% and 75% of the total weight, respectively. In the case of the fibrous fraction of alfalfa, the ratio of hemicellulose to cellulose is 1:1.9 and the sum of hemicellulose, cellulose, and solubles is approximately 72% of the total weight. The lignin concentration of the alfalfa, at 7.8% is half or less than that listed for the three groups of cellulosics. Protein at 10-11% is high relative to other cellulosics. It is conjectured that the protein may form highly insoluble complexes during the high temperature pretreatment to hydrolyze the hemicellulose. There may, therefore, be some incentive to remove more of it with the juice by means of

rewetting during the pressing process. The ash content at 8.6% is also relatively high. The value of this ash as agricultural fertilizer remains to be determined. The exact content of the solubles likewise has not yet been determined.

Alternately, the fibrous fraction could be field-dried and combusted for production of electrical power. Bomb calorimeter tests have given a higher heating value of approximately 19,000 kJ/kg. At a yield of 9.45 t/ha, this would be approximately 50,000 kWh/ha, or if converted to electricity at an efficiency of 0.3, electrical energy of approximately 15 MWh/ha.

# **Conclusions**

"Staging" of the area to be cut by starting early and spreading the first cutting over approximately 35-days, with repeat cuttings of any area at 35 day intervals, appeared to work well in 1995 and 1996. This strategy could allow harvesting and processing equipment to be used quite steadily, at near capacity, during the entire growing season. On the other hand, it should be recognized that the data presented is for only one set of unreplicated plots for two harvesting seasons. The measured yields are 125%-150% of those frequently reported. Less favorable weather, for example, could reduce yields and delay regrowth.

The high unit values and yields of certain juice products — e.g., soluble food-grade protein concentrates:  $\cong 0.6$  t/ha, particulate protein-xanthophyll concentrates  $\cong 1.6$  t/ha, industrially valuable enzymes  $\cong 5-25$  kg/ha — can make the economics of wet fractionation attractive. The potential high value of the juice products can, in a sense, "subsidize" the ligno-cellulosic fraction making it possible to market it in the neighborhood of \$40/t dry matter.

The yield and composition of the fibrous fraction would allow over 4000 liters/ha of ethanol to be produced annually at 0.85 of the stoichiometric conversion. If converted to electrical energy at an efficiency of 0.3, the result would approximate 15 MWh/ha annually.

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Table 1. Composition (%) of the fibrous fraction obtained from maceration and juice expression of alfalfa (4 plots x 4 cuttings per plot).

						Solubles
	Cellulose	Hemicellulose	Lignin	Protein	Ash	(by difference)
Mean	33.05	17.48	7.77	10.94	9.28	21.48
Std. Dev.	4.40	3.44	1.67	1.75	2.00	3.91
Max.	43.38	24.40	12.97	15.56	16.38	28.32
Min.	24.04	12.52	4.83	8.13	6.81	14.51
$n = 16 \times 2 \text{ reps}$						
t/ha*	3.12	1.65	0.74	1.03	0.81	2.09

<sup>\*</sup>Based on annual herbage yield of 13.5 t/ha and fibrous fraction = 0.7 x herbage = 9.45 t/ha.

Table 2. Potential ethanol production from alfalfa "fiber."

		Stoichiometric		
Material	t/ha	Ratio	Efficiency	Ethanol yield (t/ha)
Cellulose	3.12	.568	.85	1.51
Hemicellulose	1.65	.581	.85	0.81
Solubles	2.09	.568	.85	<u>1.01</u>
		(assumed)	Total	3.37  t/ha = 4266  liters